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Nutritional Profile of Beef on the Shelves: Influence of Production System

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Abstract: This work chemically characterized the beef that is arriving at the retailer stores, from extensive, semi-extensive and intensive systems. A total of 41 beef samples were collected over 27 months. Extensively grown beef is produced on a smaller scale and consequently is rarely found at supermarket butcheries. Total fat and protein contents, fatty acids profile, cholesterol and α-tocopherol content had shown variability along the year in meat from the same producer. The indices of thrombogenicity and atherogenicity were calculated to have more insight into the healthiness of the beef and among semi-extensive producers, there is one that stands out with a better fatty acids profile and lower thrombogenicity index than intensively grown beef. In general, the production system information by itself does not give enough insight into the beef quality on the shelf. However, intensively grown beef delivers fatter meat with a less healthy fatty acids profile than the meat semi-extensively and extensively grown in the Alentejo region.

Keywords: Beef quality; Beef production systems; Extensive production; Intensive production; Semi-intensive production

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1. Introduction

Beef is produced under extensive (E), semi-extensive (SE) or intensive (I) production systems. Extensive production is characterized by pasture feeding, longer time of animal growth and large area per animal. This type of

production improves animal welfare, environmental quality (e.g. landscape and attractiveness) and enriches biodiversity ofagro-eco-systems (Vestergaard *et al.,* 2000; Teixeira *et al.,* 2015). Intensive production involves concentrate-feeding, the time of production decreases as well as the space per animal. Consequently, antibiotics may be used, and animal welfare is usually compromised. However, it is more environmentally sustainable with less greenhouse gas emissions per kilogram of meat (Swain *et al.,* 2018). A combination between both production systems is often adopted to overcome the limitations of extensive production such as low grassland productivity (Teixeira *et al.,* 2015), related with seasonal nature of pastures, which limits feed quality and implies food shortage. This production system is named semi-extensive or semi-intensive and combines production characteristics from both extensive and intensive production systems, where feeding system combines grass and concentrate, and growth takes place in both free-range and feed-lots, the last takes place usually during a fattening period before slaughtering.

It has been widely demonstrated that extensive production of beef, provides leaner meat with healthier fatty acids profile including lower *n*−6/*n*−3 fatty acids ratio (Wood *et al.,* 2004; Menezes *et al.,* 2013; Mezgebo *et al.,* 2017; Couvreur *et al.,* 2019). Nevertheless, for animals fed on concentrate, the supplementation with oils (e.g. sunflower, linseed oil or fish oil) contributes to increase the conjugated linoleic acid (CLA) and *n*-3 polyunsaturated fatty acidscontents (Mir *et al.,* 2004; Dhiman *et al.,* 2005; Enser *et al.,* 2016). Consumer has been increasingly demanding for healthier food and believe that grass-fed animals provide healthier meat. Fat and cholesterol content have been one of the major health concerns in meat (Van Wezemael *et al.,* 2010). However, the fat reduction can have a negative impact on sensory quality of beef affecting negatively juiciness, tenderness, and flavour (Hunt *et al.,* 2016). Consumers preferred beef from grass-fed animals when evaluating production system but in sensory analysis, the concentrate-fed meat had higher classification for odour, tenderness, juiciness, taste and overall assessment (Font *et al.,* 2011; García-Torres *et al.,* 2016). Moreover, the knowledge that higher animal welfare is achieved in extensive production may be another reason for the preference for grass-fed system. Some studies had found that consumers are willing to pay more for animal welfare or environmental-friendly label (Schnettler *et al.,* 2008; Tonsor *et al.,* 2015; Sonoda *et al.,* 2018).

It is not common the evaluation of the indices of thrombogenicity (TI) and atherogenicity (AI) in beef, although it is calculated for other meat products such as burgers (Afshari *et al.,* 2017; Heck *et al.,* 2017), sausages (Romero *et*

al., 2013) and chicken products (Popova *et al.,* 2016). However, AI and TI are relevant indices that can indicate the influence of diet on coronary heart disease. AI relates the risk of atherosclerosis and is based on SFA that can increase or UFA that can decrease the level of blood cholesterol. TI values relate to the tendency to form clots in the blood vessels, defined as the relationship between the pro-thrombogenic (SFA) and the anti-thrombogenicity acids (MUFA, n-6 PUFA and n-3 PUFA) (de Alba *et al.,* 2019; Ribeiro *et al.,* 2021). Thus, in this work TI and AI were calculated to get more insights on healthiness of beef under study.

In a retailer store, beef is available from different production systems. This study aims to evaluate the nutritional quality of beef, how it is presented to the consumer, i.e. to analyse the beef that is being sold in the retailer stores, from extensive and semi-extensive beef producers from Alentejo region, Portugal, in a consumer perspective. Additionally, use a foreign intensively grown beef for comparison.

2. Materials and Methods

2.1. Animals and Production Systems

Randomly along 27 months, *Longissimus dorsi* muscle steaks from steers were collected from a retailer store, imitating the reality of consumer purchases. It was selected four different producers from the Alentejo region, Portugal. One producer performs an extensive production and the other three producers use a semi-extensive production system. The breed is common to all producers (crossbreed from Alentejo), but there are small production differences between producers. Extensive production system (E) is characterized by total growth on green natural pasture under continuous grazing until slaughter. Only two samples from this producer were achievable in store, however, given the valuable information that this sample may give, they were still analysed, and results are presented. Semi-extensive (SE) production system of the three Portuguese producers is characterized by growth on green natural pasture along the first six months of animals' lives, followed by a concentrate-fed period. The differences in the production among the three SE producers is related to the time of fattening and concentrate (commercial concentrates), which were not of our knowledge. For comparison, foreign intensively grown beef was also collected at the same store in the same conditions. Table 1 summarizes the mean carcasses weights and SEUROP classifications for all the producers.

Table 1: Mean weight and SEUROP classification of the carcasses for all producers

Producer	*Carcass weight (kg)*	*SEUROP carcass rating*	*SEUROP fat rating*
SE1	3431± 198[a]	R	2 to 3
SE2	3423 ± 298[a]	U to R	2 to 3
SE3	3875 ± 154[b]	U to R	2 to 3
E	3395 ± 263[a,b]	R	2
I	3861 ± 103[a,b]	Mainly U	2 to 3

[a,b]: Means within the same column having no superscript letters in common differ (p< 005) SD is the standard deviation of the means

2.2. Sample Collection and Preparation

Samples were collected in a retailer store assuring traceability of samples. At least 200 g steaks from *Longissimus dorsi* muscle were transversely cut, vacuum packaged at the cutting plant and sent to the retailer store. After collection at the retailer store, samples were cut in pieces and homogenized in a meat mincer before weighing for each analysis.

2.3. Chemicals

Dimethylformamide (DMF), methanolandn-hexane were HPLC grade and sulphuric acid (H_2SO_4) was analytical grade (VWR Scientific, Carnaxide, Portugal). ABTS diammonium salt (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetic acid (min. 99.7%), acrylamide (99%), ammonium persulfate (APS, min. 98%), EDTA (99.0-101.0%), N,N'-methylene-bisacrylamide (99.5%), potassium chloride (min. 99.5%), sodium methoxide (95%), Supelco 37 mix, BAME mix, Tetramethylethylenediamine (TEMED, approx.. 99%), trichloroacetic acid (TCA, min. 99%) and Tris (hydroxymethyl) aminomethane were obtained from Sigma (Sigma-Aldrich, Sintra, Portugal). Tritridecanoin (99%) was from Nu-Chek Prep, Inc. (Elysian, MN, USA). It was used ultrapure sodium dodecyl sulfate (SDS, min. 99.0%) from Applichem, PANREAC. Potassium hydroxide and sodium hydroxide were from EKA (Azko Nobel Company, Laborspirit, Lisboa, Portugal). It was used 32% hydrochloride acid and potassium persulfate from Merck (Merck S.A., Portugal). Glycerol (99.95%) was obtained from Fisher Scientific, UK. Tricine Sample Buffer from BIO-RAD (Portugal) used to perform SDS-PAGE was composed for 200 mM Tris-HCl, pH 6.8, 40% glycerol, 2% SDS and 0.04% Coomassie Blu G-250.

2.4. Proximate Composition

Total protein content was determined by the Kjeldahl method (Qualidade, 2006) using a Kjeltec system 1002 distilling unit (Tecator; Hoganas, Sweden).

The conversion factor used was 6.25. The total intramuscular lipid content was determined by Soxhlet method, after hydrolysis with 4N hydrochloric acid and extraction with petroleum ether (Qualidade, 1979). Dry matter and ashes were determined according to NP1614-1:2009 (Qualidade, 2009) and NP1615:2002 (Qualidade, 2002) Portuguese standards respectively. The pH value was measured after homogenization of 1 g of minced meat with 10 ml of 0.1 M KCl solution, using a *Crison micro pH2002* pH meter (Qualidade, 2008).

2.5. Fatty Acids Profile

Fatty acids were quantified after derivatization to FAMEs according to Pimentel *et al.* (2015). 100 µl of tritridecanoin (1.50 mg/ml) was added to 250 mg of minced fresh meat and 2.26 ml of methanol were added, followed by 1 ml of hexane and 240 µl of sodium methoxide in methanol (5M). Samples were vortexed and incubated at 80 ºC for 10 min. After cooling in ice, 1.25 ml of DMF and 1.25 ml of sulphuric acid in methanol (3M) were added. Samples were vortexed and incubated at 60 ºC for 30 min. Finally, after cooling, 1 ml of hexane was added, and the samples were subsequently vortexed and centrifuged (1250 g; 18 ºC; 5 min.). The fatty acidmethyl esters (FAME) in the upper layer were collected for further analysis.

FAME extracts were analysed in a gas chromatograph HP6890A (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionization detector (GLC-FID) and aBPX70 capillary column (60 m x 0.32 mm x 0.25 µm; SGE Europe Ltd, Courtaboeuf, France). Analysis conditions were as follows: injector temperature 250 °C, split 25:1, injection volume 1 µL; detector (FID) temperature 275 °C; hydrogen was the carrier gas at 20.5 psi; oven temperature program: started at 60 °C (held 5 min), then raised at 15°C/min to 165 °C (held 1 min) and finally at 2°C/min to 225 °C (held 2 min). Supelco37 mix were used for identification of fatty acids. GLC-Nestlé36 was assayed for calculation of response factors.

2.5.1. Nutritional Quality Indices

Nutritional fatty acids quality indices were analysed from fatty acids composition data. The indices of thrombogenicity (*TI*) and atherogenicity (*AI*) were calculated using Eqs. (1) and (2), respectively.

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{\left[0.5 \times (\Sigma MUFA + \Sigma n6) + 3 \times \Sigma n3 + \frac{\Sigma n3}{\Sigma n6}\right]} \qquad \text{Equation (1)}$$

$$AI = \frac{[C12:0 + 4 \times C14:0 + C16:0]}{[\Sigma MUFA + \Sigma PUFA]}$$ Equation (2)

2.6. Protein Profile

2.6.1. Protein Extraction

Protein profile was assessed using SDS-PAGE and Fast Protein Liquid Chromatography (FPLC) techniques, after protein extraction according to Claeys *et al*. (1995). Briefly, 2.5 g of minced thawed meat was homogenized in 25 ml of a buffer solution (pH 7.6, 4°C) containing 0.25 M sucrose, 0.05 M Tris and 1 mM EDTA using an Ultra-Turrax. The homogenate was centrifuged at 4000 rpm, 5 min, 4ºC. The supernatant was decanted and filtrated, and the pellet was resuspended in 25 ml of buffer solution (pH 7.6 at 4°C) containing 0.05 M Tris and 1 mM EDTA. Centrifugation was repeated in the same conditions and the supernatant was filtrated. The procedure is repeated with 25 ml of a 0.15 M KCI solution (4°C). The three supernatants were collected.

2.6.2. Fast Protein Liquid Chromatography (FPLC)

For molecular weight assessment of the supernatants (sarcoplasmic proteins), the collected supernatants were centrifuged, and an aliquot of 100 µL was injected into the FPLC. The proteins were separated by gel filtration chromatography using a Superdex 200 10/300 GL column connected in series to a Superdex Peptide 10/300 GL column (GE Healthcare Life Sciences, Freiburg, Germany), coupled to an FPLC AKTA-purifier system (GE Healthcare Life Sciences, Freiburg, Germany). The eluent used was 0.05 M phosphate buffer pH 7.0, containing 0.15 M sodium chloride (ionic strength) and 0.2 g/L of sodium azide (as preservative) at a flow rate of 0.5 ml/min. Elution was monitored at 280 nm and approximate molecular mass of protein solutions were determined with standard protein Gel Filtration Calibration Kits (GE Healthcare Life Sciences, Freiburg, Germany) in the range 10 kDa to 669 kDa.

2.6.3. SDS-PAGE

SDS-PAGE was performed for both pellet (myofibrils) and supernatant (soluble proteins) protein profile analysis. 100 mg of the pellet was resuspended in 980 µl of Tricine Sample Buffer (Bio-Rad, Portugal) and 20 µl of 2% dithiothreitol (DTT). The supernatant (500 µl) was homogenized with the same sample buffer (480 µl plus 20 µl of 2% DTT) in a 1:1 ratio. Samples were then heated at 100ºC for 3 min, centrifuged 14000 rpm, for 5 min and decanted.

Electrophoresis was carried out in a Criterion™ Vertical Electrophoresis Cell (BIO-RAD, Portugal). It was prepared a 8% acrylamide separation gel and a 3% acrylamide stacking gel, using 30% gel monomer (acrylamide/ N,N'-methylene-bisacrylamide 29:1). Gel thickness was 1.0 mm. It was used a Tris-Tricine pH 8.3 running buffer and a pre-stained protein marker (GRS Protein Marker MultiColour, GRISP) for monitoring protein separation during SDS-PAGE. The separation was started with a current of 75 V for 5 minutes and then increased to 150 V for more 35 to 45 min until the protein marker were totally separated.

After running, gels were immersed in 10% TCA solution for fixing bands for 1 h, followed by stain solution containing 0.25% Coomassie Blue R-250, 10% acetic acid and 50% methanol overnight. For gel destaining, it was used a 10% acetic acid and 25% methanol destain solution for about 8 h. The gels were imaged using a ChemiDoc XRS+ system with a White Light Conversion Screen (BIO-RAD).

2.7. Vitamin E and Cholesterol Content

α-tocopherol and cholesterol contents were determined according to Mestre Prates *et al.* (2006). To 0.75 g of fresh meat, it was added 0.20 g of *L*-ascorbic acid and 5.5 ml of ethanolic KOH (11%, w/v), followed by homogenization through vortex. Air was replaced by nitrogen and the mixture was vortexed again until total dissolution of ascorbic acid. Afterwards, samples were heated in a water bath at 80 °C for 15 min, 200 rpm. Then, the mixture was cooled under tap water for 1 min. 1.5 ml of distilled water and 3 ml of *n*-hexane (25 μg/ml BHT) were added and mixed in a vortex for 1 min. Followed by centrifugation 1500 *g*, 5 min. An aliquot of exactly 1 ml was collected to a vial for HPLC analysis.

A BECKMAN System Gold® linked to a Waters™ 474 Scanning Fluorescence Detector (excitation wavelength of 290 nm and emission wavelength of 320 nm) and a Diode Array Detector (DAD) 168 Detector (210 nm) with a VARIAN ProStar Model 410 AutoSampler was used to α-tocopherol and cholesterol chromatography using a normal-phase silica column (Kromasil 60-5-SIL, 250 mm, 4.6 mm ID, 5 μm particle size. The mobile phase was 1% v/v isopropanol in *n*-hexane with a flow rate of 1 ml/min. The total run time was 20 min and the injection volume was 20 μl. Standard curves of peak area vs. concentration were used for each compound quantification.

2.8. Antioxidant Capacity

The phenolic compounds were extracted according to Mahayothee *et al.* (2016) method. Thawed minced meat (20 g) was mixed with 100 ml of methanol using

a T18 digital ULTRA-TURRAX®, and the mixture was kept in agitation for 3h, in dark, at room temperature. Then, the mixture was filtrated and methanol was evaporated using a rotary evaporator (Rotavapor R–210, Buchi, Switzerland) until a final volume of 10 ml.

The water-soluble antioxidant capacity assay was performed according to Gião *et al.* (2007). The $ABTS^{\bullet+}$ solution was obtained by the reaction between 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate solutions (at 1:1, v/v), during 16 h in dark with agitation. The $ABTS^{\bullet+}$ solution was diluted with methanol until 0.700 ± 0.020 of absorbance at 734 nm (measured with a Shimadzu 1240 UV–visible spectrophotometer). For sample analysis, 10 µl of the extract was mixed with 1ml of $ABTS^{\bullet+}$ diluted solution and the absorbance was read after 6 min of reaction in dark with agitation. Ascorbic acid was used as standard, and the results were expressed as µg of ascorbic acid equivalents per g of fresh meat.

DPPH assay was based on the method of Brand-Williams *et al.* (1995). The extract (100 µl) was mixed with 900 µl of 0.06 mM DPPH solution and kept in dark with agitation at room temperature. After 2 h, the absorbance was read at 515 nm. Trolox was used as standard and results were expressed as µg of Trolox equivalents per g of fresh meat.

For both methods (ABTS and DPPH), a blank was performed using methanol and the % of inhibition was calculated as % inhibition = $(A_0 - A_S / A_0) \times 100$. Where A_0 and A_S are the absorbance of the blank and the sample, respectively.

2.9. Statistical Analysis

When data followed a normal distribution,it was performed a One-way analysis of variance (ANOVA) followed by Tukey HSD's post hoc test at the $p< 0.05$ significance level when data meet the homogeneity of variances assumption. When data did not meet the homogeneity of variances assumption, Games-Howell post hoc test was performedat the $p< 0.05$ significance level. For data with no normal distribution, the Kruskal-Wallis non-parametric test was performed at the $p< 0.05$ significance level. When significant differences were found, the Mann-Whitney test was performed in pared samples at the $p< 0.05$ significance level.A Principal Component Analysis (PCA) was performed to the nutritional parameters, using a varimax rotation method with Kaiser normalization. SPSS version 22 software was used to perform all the statistical analyses.

3. Results and Discussion

3.1. Chemical Composition

The results of the chemical composition of the samples are presented in Table 2, presenting the mean for each producer. Moisture content was approximately 74% for SE and E production systems and 72% for I production system, being different ($p< 0.05$) between SE1 and I as well as between E and I. Contrastingly, total intramuscular fat amount was higher in I meat than it was in SE and E meats, differing ($p < 0.05$) between SE1 and I and SE2 and I. Previous studies also indicated that higher energy intake resulted in higher amount of intramuscular fat and correspondingly lower moisture content (Mezgebo *et al.*, 2017). Production system (Vestergaard *et al.*, 2000), as well as animal performance during stocking (Neel *et al.*, 2007), influence the fat content in beef. Intensively grown young bulls presented higher amount of intramuscular fat than extensively grown young bulls, independently of the slaughter weight and the muscle (*M. semitendinosus*, *M. longissimus* and *M. supraspinatus*) (Vestergaard *et al.*, 2000). The two samples/animals from E production had very different results regarding fat content, with 4.07±0.16 (% ± SD) and 0.46±0.12(% ± SD). This shows that this production system is less consistent and the nutritional composition of beef produced under this production system can vary significantly even when produced by the same producer. The uncertainty in the feed system may be the cause of this variability, as the two samples are from different times of the year. The sample with higher amount of fat corresponds to slaughter in winter (February) and, the sample with the lower amount of fat had a slaughter time in autumn (November).

Table 2: Chemical composition of *longissimus dorsi* muscle by production system (SE – semi-extensive production system. E – extensive production system. I – intensive production system)

	Moisture (% ± SD)	*Total fat (% ± SD)*	*Total protein (% ± SD)*	*Ash (% ± SD)*	*pH ± SD*
SE 1 (*n*=14)	73.86 ± 1.14 [a]	2.65 ± 1.01 [a]	23.29 ± 1.14 [a]	1.09 ± 0.05[a]	5.74 ± 0.42[a]
SE 2(*n*=7)	73.46 ± 0.89[a,b]	3.29 ± 1.02 [a]	22.67 ± 0.88 [a]	1.07 ± 0.04 [a]	5.76 ± 0.34[a]
SE 3 (*n*=6)	73.52 ± 1.67[a,b]	3.22 ± 1.73[a,b]	24.05 ± 1.57 [a]	1.04 ± 0.04 [a]	5.63 ± 0.18[a]
E (*n*=2)	74.82 ± 1.34 [a]	2.26 ± 2.55[a,b]	23.56 ± 0.70 [a]	1.01 ± 0.02 [a]	5.61 ± 0.04[a]
I (*n*=12)	71.66 ± 1.70 [b]	5.37 ± 2.31 [b]	23.16 ± 1.39 [a]	1.08 ± 0.07 [a]	5.62 ± 0.21[a]

[a,b]: Means within the same column having no superscript letters in common differ ($p< 0.05$).SD is the standard deviation of the means.

3.2. Fatty Acids Composition

Fatty acids profile of beef is strongly affected by the feeding system (Wood *et al.*, 2004; Dhiman *et al.*, 2005) and have a very important role in human health (Nuernberg, 2009). A high *n*-6/*n*-3 ratio increases the risk of obesity but on the other hand, increasing the intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) the previous effect of the high*n*-6/*n*-3 ratio can be reversed (Simopoulos, 2016).

In this study, it was found that for different production systems, SE, E and I, the *n*-6/*n*-3 ratiowas not different in mean ($p \geq 0.05$) (Table 3). However, for E producer, the variability between samples, shows that this kind of production may result in a very low *n*-6/*n*-3 ratio: 4.29 ± 0.05 as well as aratio of 15.86 ± 1.26. This variability may be explained by the season of the animal's slaughter, but it was not statistically observed any season effect for all samples (data not shown), however, the lack of evidence of season effect may be related to the low number of samples per season, especially for E producer. Previous studies had shown that cooler seasons result in higher *n*-6/*n*-3 ratio as found in this work (higher value corresponds to the sample from winter, and the lower value corresponds to the sample from autumn, which is in agreement with previous works with values for autumn) (Pestana *et al.*, 2012).

Eicosapentaenoic acid (EPA C20:5*n*3) is one of the*n*-3 fatty acids with most protective effect for cardiovascular health (Nuernberg, 2009) and it was found an apparent higher amount of these compounds in SE1 and E producers, however, significant differences were not found ($p \geq 0.05$). It was found the same tendency for linolenic acid (C18:3 c9c12c15), where, in mean, it was found the higher values in SE1 and E, but once there is high variability among animals in these producers, there are no significant differences in comparison to the other producers ($p \geq 0.05$). Conjugated linoleic acid (CLA) has anticarcinogenic and antiatherogenic properties and reduces body fat while promoting lean body mass (Azain *et al.*, 2000; Tsuboyama-Kasaoka *et al.*, 2000). In this study, it was verified a significant difference ($p = 0.001$) between SE1 and I production systems with a higher amount in I producer. Producer SE3 had a higher percentage of CLA than I producer, but with no significant difference ($p \geq 0.05$). Previous studies concluded that grain-fed animals, when supplemented with linoleic acid–richoils (e.g. sunflower and linseed oil), had a greater amount of CLA comparing with animals fed only on grain or concentrate (Mir *et al.*, 2004; Dhiman *et al.*, 2005).

The sum of saturated fatty acids (SFA) was lower for SE2 in comparison to SE1 and I producers ($p < 0.05$). Previous studies comparing grain- and grass-fed

steers, had different results between them (Daley *et al.*, 2010). For some studies, it was found a higher amount of SFA in grain-fed steers but in other studies, the higher amount of SFA was found in the grass-fed animals (Daley *et al.*, 2010). As well in this study, only one out of three semi-extensively grown beef presented a differently lower amount of SFA in comparison to the intensively grown steers.

The higher amount of polyunsaturated fatty acids (PUFA) was found in E producer, which was significantly higher ($p< 0.05$) than the amount found in I producer. The unsaturated fatty acids (UFA) and SFA ratio, presented in Table 3, was higher for SE2 in comparison with SE1 and I producers ($p = 0.001$ and 0.010 respectively), despite the fact that for the sum of both monounsaturated fatty acids (MUFA) and PUFA there are no differences between SE2 and I. The amount of saturated fatty acids is very low for SE2 (43.98 ± 2.69) comparing with SE1 and I producers (49.63 ± 2.75 and 48.67 ± 2.54, respectively).

Overall, SE2 is apparently the production system providing the best fatty acids profile for human health, containing lower content in SFA, a higher amount of CLA (although not statistically different from SE3, E and I producers), higher UFA/SFA ratio and lower TI (see Table 3). Results showed significant differences in TI between the sample SE2 and I, but no differences among the other producers. As TI relates to the tendency to form clots in the blood vessels, TI is higher for the most thrombogenic foods (Ulbricht and Southgate, 1991). The value of TI being lower for SE2 beef reinforces the idea that the SE2 production system provides healthier meat. TI values ranged between 1.39 (SE2) and 1.70 (I) which are higher than fish and vegetable oils TI values (TI values of 0.25-0.32) (de Alba *et al.*, 2019) and olive oil by-products (TI value of 0.39) (Ribeiro *et al.*, 2020). There were no significant differences in the AI values, which ranged between 0.58 (SE2) and 0.71 (I). The highly atherogenic food reported was coconut oil with AI value of 13.63 and foods with the lowest AI values were mackerel, olive and sunflower oil with 0.28, 0.14 and 0.07 AI values respectively (de Alba *et al.*, 2019).

Table 3: Intramuscular fatty acid composition of *longissimus dorsi* muscle (g/100 g fatty acids) by production systems

Sample	*SE1* *(n=14)*	*SE2* *(n=6)*	*SE3* *(n=6)*	*E* *(n=2)*	*I* *(n=12)*
C14	2.93 ± 0.73[a]	2.31 ± 0.54[a]	2.42 ± 0.55[a]	2.23 ± 0.77[a]	2.61 ± 0.31[a]
C16	23.07 ± 1.90[a]	22.63 ± 0.85[a]	21.96 ± 3.20[a]	21.66 ± 2.44[a,b]	25.76 ± 2.15[b]
C16:1[(A)]	2.61 ± 0.74[a]	3.50 ± 0.91[a]	3.07 ±0.68[a]	2.31 ± 1.26[a]	3.05 ± 0.54[a]

Sample	*SE1* (*n*=14)	*SE2* (*n*=6)	*SE3* (*n*=6)	*E* (*n*=2)	*I* (*n*=12)
C18	18.80 ± 2.97[a]	14.18 ± 1.92[b]	16.76 ± 3.81[a,b]	19.64 ± 5.03[a,b]	16.32 ± 1.91[a,b]
C18:1[(B)]	34.17 ± 4.25[a]	41.43 ± 3.83[b]	38.57 ± 4.43[a,b]	29.65 ±12.04[a,b]	39.32 ± 3.39[b]
C18:2	8.89 ± 2.47[a]	7.49 ± 1.73[a]	7.23 ± 2.59[a]	10.42 ± 3.64[a]	6.34 ± 2.73[a]
C18:3 c9c12c15	0.44 ± 0.65[a]	0.22 ± 0.05[a]	0.20 ±0.07[a]	0.88 ± 0.96[a]	0.18 ± 0.08[a]
C18:2 c9t11	0.18 ± 0.1[a]	0.21 ± 0.14[a]	0.23 ± 0.05[a]	0.18 ± 0.01[a]	0.26 ± 0.08[a]
CLA c,c	0.06 ± 0.01[a]	0.08 ± 0.01[a,b]	0.14 ± 0.15[a,b]	0.06 ± 0.03[a,b]	0.09 ± 0.02[b]
C20:1 c9	0.14 ± 0.03[a]	0.18 ± 0.04[a]	0.17 ± 0.04[a]	0.09 ± 0.05[a]	0.20 ± 0.08[a]
C20:4 *n*6	2.06 ± 0.64[a,d]	1.34 ±0.46[a,c]	1.23 ± 0.76[a,c]	3.35 ± 1.19[d]	0.92 ± 0.60[c]
EPA C20:5 *n*3	0.17 ± 0.19[a]	0.07 ± 0.06[a]	0.09 ± 0.05[a]	0.51 ± 0.59[a]	0.05 ±0.03[a]
DPA C22:5 *n*3	0.36 ± 0.26[a]	0.21 ± 0.10[a]	0.21 ± 0.10[a]	0.93 ± 0.81[a]	0.16 ± 0.09[a]
ΣSFA[(C)]	49.63 ± 2.75[a]	43.98 ± 2.69[b]	47.07 ± 3.87[a,b]	49.86 ± 4.62[a,b]	48.67 ± 2.54[a]
ΣMUFA[(D)]	37.72 ± 4.82[a]	46.08 ± 4.09[b]	43.37 ± 5.68[a,b]	32.90 ±13.15[a,b]	43.30 ± 3.77[b]
ΣPUFA[(E)]	12.65 ± 4.11[a]	10.11 ± 2.40[a,b]	9.56 ± 3.86[a,b]	17.23 ± 8.54[a]	8.04 ± 3.53[b]
Total *n*-6[(F)]	10.88 ± 3.00[a]	8.75 ± 2.07[a,b]	8.32 ± 3.36[a,b]	13.71 ± 4.80[a,b]	7.17 ± 3.29[b]
Total *n*-3[(G)]	0.98 ± 1.07[a]	0.50 ± 0.18[a]	0.50 ± 0.20[a]	2.32 ± 2.36[a]	0.40 ± 0.17[a]
n-6/*n*-3 ratio	16.84 ± 7.21[a]	18.77 ± 5.56[a]	17.33 ± 4.42[a]	10.08 ± 8.18[a]	19.65 ± 8.05[a]
UFA/SFA ratio	1.02 ± 0.11[a]	1.29 ± 0.12[b]	1.15 ± 0.19[a,b]	1.02 ± 0.19[a,b]	1.06 ± 0.11[a]
TI	1.66± 0.22[a,b]	1.39 ± 0.16[a]	1.52 ± 0.34[a,b]	1.43 ± 0.08[a,b]	1.70 ± 0.18[b]
AI	0.69± 0.10[a]	0.58 ± 0.07[a]	0.60 ± 0.13[a]	0.61 ± 0.05[a]	0.71 ± 0.08[a]

Results are represented as mean ± SD.

[a, b, c, d] Means within the same line having no superscript letters in common differ (p< 0.05). SD is the standard deviation of the means.

[(A)]Corresponds to the sum of C16:1 t9, C16:1 c7 and C16:1 c9

[(B)] Corresponds to the sum of C18:1 t6-t9, C18:1 t10, C18:1 t11, C18:1 c14-t16, C18:1 c9, C18:1 t15, C18:1 t12, C18:1 c11, C18:1 c12 and C18:1 c13

[(C)] The sum of saturated fatty acids was calculated as: C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C24:0.

[(D)] The sum of monounsaturated fatty acids was calculated as: C14:1 + C15:1 + C16:1 (corresponding to the sum of C16:1 t9,C16:1 c7andC16:1 c9) + C17:1 (corresponding to the sum of C17:1 c9 and C17:1 c10) + C18:1 (corresponding to the sum of C18:1 t6-t9, C18:1 t10, C18:1 t11,C18:1 c14-t16, C18:1 c9, C18:1 t15, C18:1 t12, C18:1 c11, C18:1 c12 and C18:1 c13) + C20:1 c9.

[(E)] The sum of polyunsaturated fatty acids was calculated as: C16:2 c9c12 + C18:2 (corresponding to the sum of C18:2 t9t12, C18:2 c9t12, C18:2 t9c12, C18:2 c9c12, C18:2 c9c15, C18:2 c9t11and CLA c,c), C18:3 c9c12c15 + C20:4 *n*6 + EPA C20:5 *n*3 + DPA C22:5 *n*3.

[(F)]The sum of *n*-6 fatty acids was calculated as: C18:2 + C20:4*n*-6.

[(G)] The sum of *n*-3 fatty acids was calculated as: C18:3 c9c12c15, EPA C20:5 *n*-3 and DPA C22:5 *n*-3. UFA corresponds to the sum of MUFA's and PUFA's.

TI, index of thrombogenicity. AI, index of atherogenicity.

PCA analysis for fatty acids profile (presented in Fig. 1) using the mean of the samples to perform the PCA showed that, in mean and not considering the variability of samples, the sample from E producer is richer in health-protective compounds such as C18:3 c9c12c15, docosapentaenoic acid (DPA), EPA and PUFA in general. This is an expected result as previous studies have been demonstrated that pasture-fed animals have a higher amount of C18:3 fatty acids and PUFA (Wood *et al.,* 2004). SE1 is the sample with higher amount of SFA. This analysis also emphasizes that SE2 sample presented the best UFA/SFA ratio. Additionally, the sample I showed a higher amount of the SFA C16:0.

3.3. *Protein Profile*

SDS-PAGE (Fig. 1) and FPLC (Fig. 2) assays were performed for several samples from each producer. These analyses demonstrated a similar protein profile between production systems. No differences were found between samples from semi-extensive, extensive and intensive production systems. As expected, myofibrils such as myosin and actin were presented in higher amount (Fig. 2A) as they are the most important proteins in the myofibrillar structure of the muscle (Tornberg, 2005). SDS-PAGE has been used as a technique to identify meat quality problems. For example, triosephosphate isomerase, creatine kinase, phosphorylase and myokines are sarcoplasmic proteins that are found precipitated appearing in the myofibrils fraction for PSE (pale, soft, exudative) meats; and, for RSE (reddish-pink, soft, exudative) meat it only appeared phosphorylase (Joo *et al.,* 1999). In this work, meat was frozen until protein extraction and SDS-PAGE analysis. Thus, as this was thawed meat, it is possible that the band number 5 in the soluble fraction (Fig. 2B) is phosphorylase. Previous works have found that in low pH (5.69 to 5.80) this band appears with greater intensity immediately bellow α-actinin than for higher pH value (Pietrzak *et al.,* 1997), so meat with higher pH values may lead to higher precipitation of this protein to soluble fraction.

The soluble fraction (Fig. 2B) has a higher amount of proteins, as meat have about 100 different proteins in sarcoplasmic fraction (Tornberg, 2005). FPLC allow the verification of lower molecular weight peptides than SDS-PAGE. The resulted chromatograms are represented in Fig. 3 and show the presence of the peptides with a molecular weight between approximately 937 Da and 1 Da. This chromatography also shows the presence of several proteins (overlapping) in the first fraction with molecular weight from 373 kDa to 20 kDa.

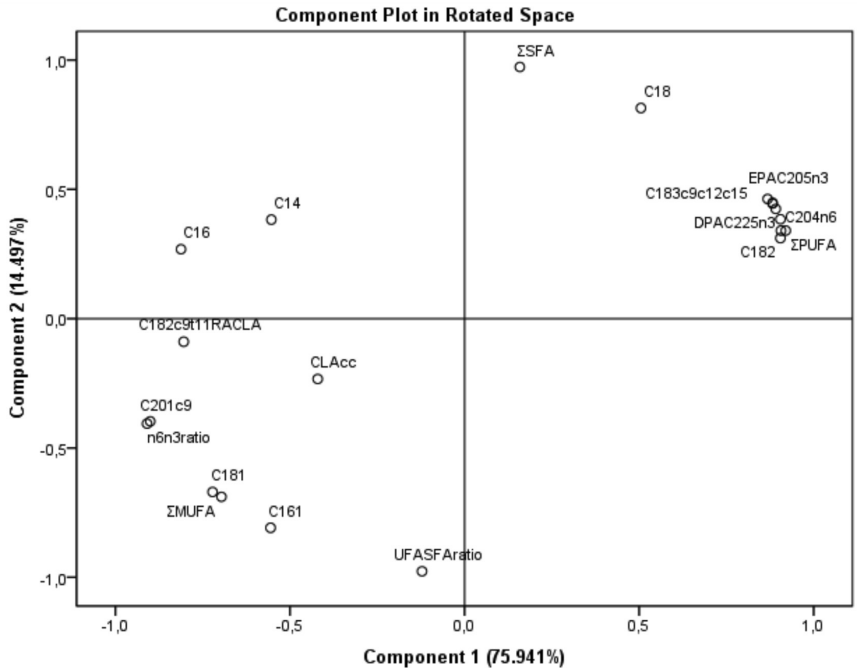

Figure 1: PCA for fatty acids Component 1 explained 74941% of the variance and Component 2 explained 14497% of the variance

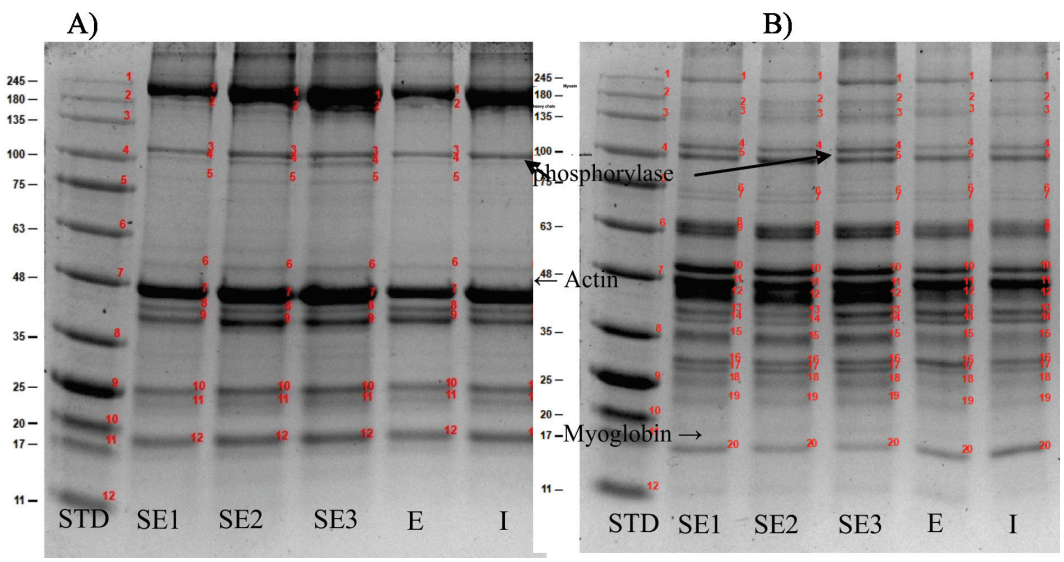

Figure 2: SDS-PAGE gels imagesof myofibrils (A) and soluble proteins (B) STD column corresponds to protein marker standard Molecular weights are presented in kDa

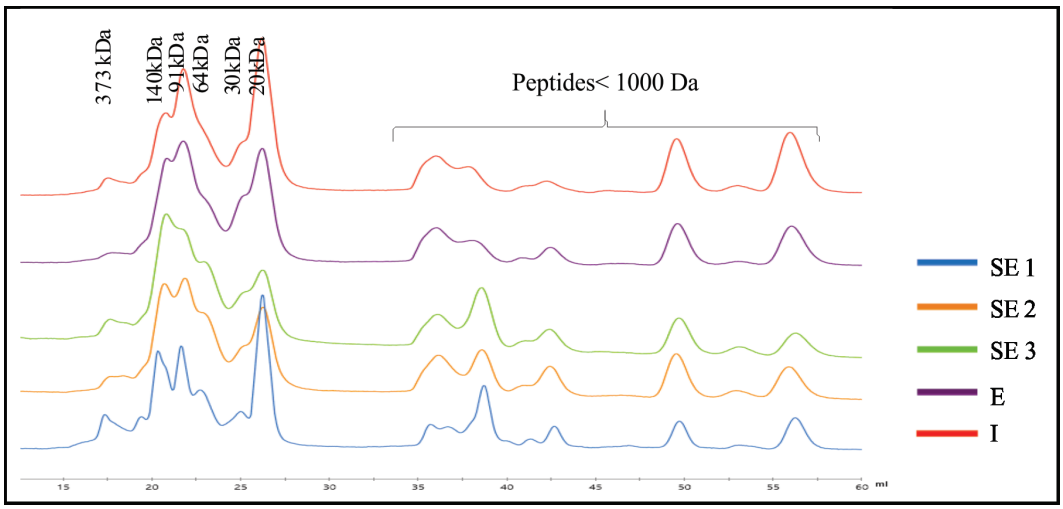

Figure 3: FPLC chromatograms for the soluble fraction of protein extraction for each production system

3.4. Vitamin E and Cholesterol Content

The total cholesterol and α-tocopherol contents in *longissimus dorsi*, from different production systems, are presented in Fig. 4. The α-tocopherol content was lower for SE3 (1.18 µg/g fresh meat), I (1.55 µg/g fresh meat) and SE2 (1.67 µg/g fresh meat), and higher for E producer (2.88 µg/g fresh meat). These results are in agreement with previous studies that had found α-tocopherol values between 0.79 and 4.07 µg/g fresh meat for steers (Scollan *et al.,* 2014). The higher α-tocopherol content values correspond to pasture-fed steers comparing with concentrate-fed or pasture-fed with concentrate or grain finishing beef (Descalzo *et al.,* 2005; Insani *et al.,* 2008; De la Fuente *et al.,* 2009; Mahecha *et al.,* 2009). However, the means were not significantly different ($p \geq 0.05$) between production systems. Previous studies have shown that α-tocopherol content is more related to preslaughter stress than with animals diet (an increase on pre slaughter stress decreases α-tocopherol content) (Delosière *et al.,* 2020).

It has been proved that the risk of obesity and hypercholesterolemia increases with the cholesterol intake, thus increasing the predisposition to several chronic diseases of the circulatory system (Mestre Prates *et al.,* 2006). The beef studied in this work provided 338-435 µg/g fresh meat of total cholesterol. Significant differences between production systems were not observed ($p \geq 0.05$). The two samples from E producer had a very different result between

each other, which shows that the variability of this production is also reflected on cholesterol. Similarly, to fatty acids profile, the producer SE2 had (with no significance) a lower amount of cholesterol, reinforcing the idea of a healthier profile in this production system.

3.5. Antioxidant Capacity

Antioxidant activity was assessed via ABTS and DPPH methods, results are presented in Fig. 5. Results from ABTS analysis showed a higher antioxidant activity for SE3 sample in comparison to SE1 and I samples. These differences ($p < 0.05$) between SEsamples as well as between SE and I samples, and, additionally the similarity between SE and E, lead to the conclusion that the production system did not affect the antioxidant activity. The results from the DPPH method were not different between all production systems, which corroborate this conclusion. As well, a recent study demonstrated that preslaughter stress has an effect on antioxidant status of meat independently of the diet of the animals (Delosière *et al.*, 2020). In addition, lipid oxidation

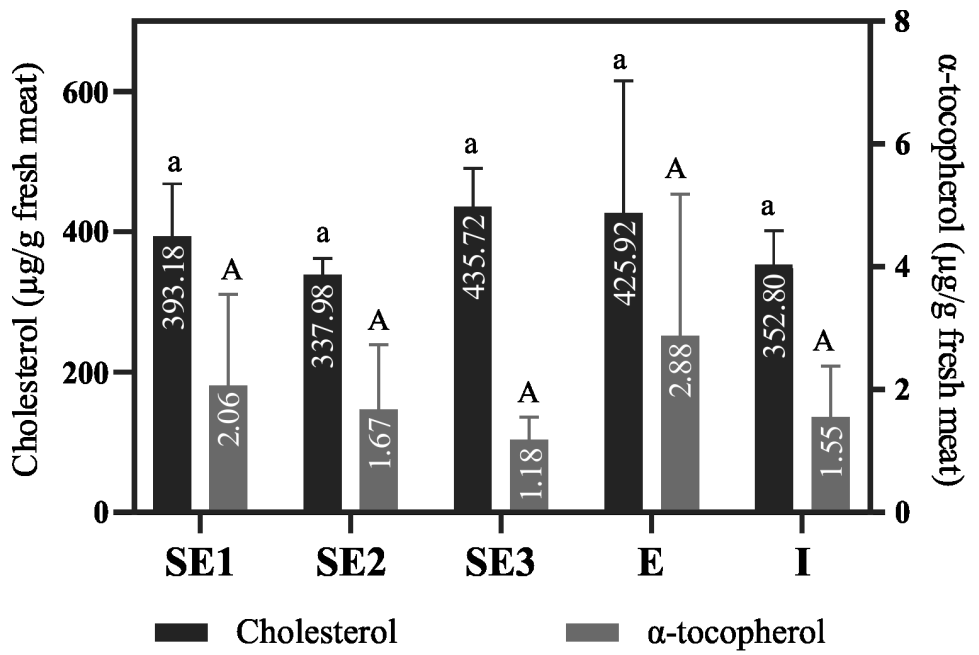

Figure 4: Mean cholesterol and α-tocopherol contents of *longissimusdorsi* from different production systems Means with superscript letters in common did not differ ($p \geq 0.05$) Error bars correspond to the standard deviation of the means Number of observations for cholesterol: SE1, n=7; SE2, n=5; SE3, n=4; E, n=2; I, n=6 (cholesterol not measured in the first year) Numberofobservations forα-tocopherol: SE1, n=11; SE2, n=5; SE3, n=6; E, n=2; I, n=9

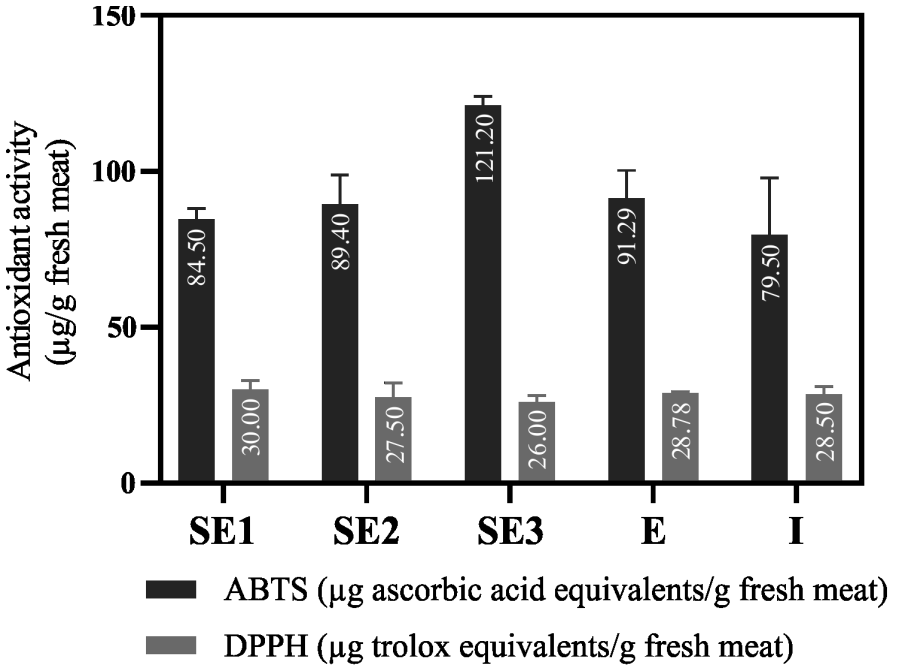

Figure 5: Mean antioxidant activity of *longissimusdorsi* from different production systems Means with superscript letters in common did not differ ($p \geq 0.05$) Error bars correspond to the standard deviation of the means Numberofobservations: SE1, n=10; SE2, n=7; SE3, n=5; E, n=2; I, n=10

results (TBA index measured in mg of malondialdehyde/kg fresh meat - data not shown) were not affected by the production system and were not correlated with antioxidant activity data.

3.6. Principal Component Analysis

A PCA was performed for the factors where significant difference between production systems ($p < 0.05$ by ANOVA) was detected. And its results are presented in Fig. 6. This analysis showed that intensively grown beef (I) was distinguished from the SE and E mainly because of its higher amount of total fat content and lower contents of C20:4 *n*-6, total *n*-6, total PUFAs and moisture. Although, it was not different from SE2 and SE3 ($p > 0.05$) in almost all these factors (Tables 2 and 3). SE2 sample also grouped together (with exception of one sample) in the negative side of Principal Component (PC) 2, which emphasizes that this production system provided beef with higher UFA/SFA ratio as well as the lower amount of SFA. SE1 samples also created a group positioned in the same location of C18 and in the opposite side of MUFA and

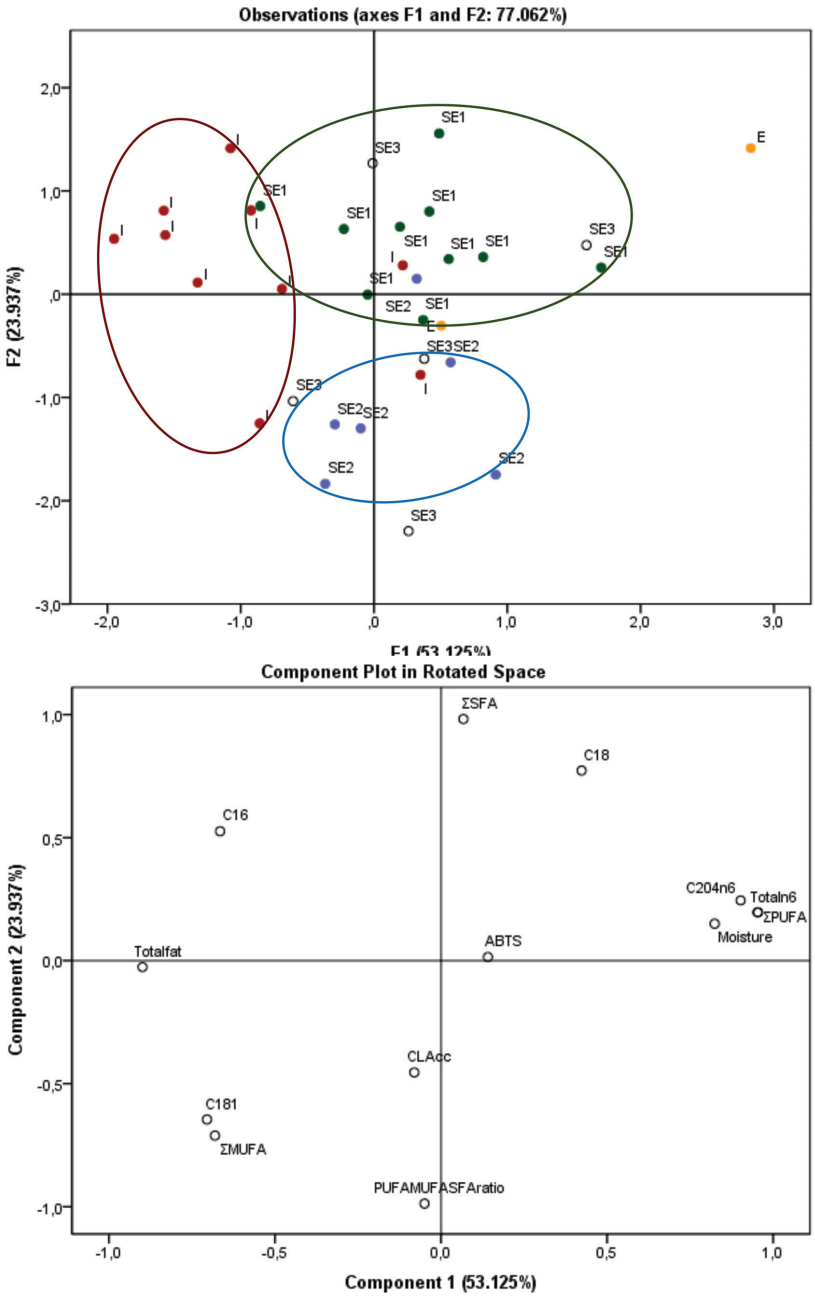

Figure 6: PCA for factors where a difference (p< 005 by ANOVA) was detected between production systems Component 1 explained 53.125% of the variance and Component 2 explained 23.937% of the variance

C18:1, and evaluating the results in Table 3 it is confirmed that this production system provided beef with higher amount of C18:0 and a lower amount of MUFA and C18:1. PCA also allowed to confirm that SE3 was not different from all the other production system, with samples located together with SE1 and SE2. The two samples from the extensive production system (E) were differently positioned, which highlighted the different results between these two samples. One E sample had similar results to the SE producers, and the other sample was detached from the other samples in the opposite quadrant of C18:1 and MUFA; and was pulled over to the positive side of PC1 with the weight of C20:4 *n*-6, total *n*-6, total PUFAs and moisture. SFA and C18:0 contents are contributing to the positioning of this sample in the positive PC2 (Fig. 6 and Table 3).

4. Conclusions

The low number of samples per producer, especially for E producer, constrained the significance of the season effect that could not be evaluated because of the low number of samples per season, which could have explained some results. Nevertheless, the results are in accordance with previous literature, and the aim of this study was accomplished, it was evaluated the quality of beef that consumer is buying at the store along the year. Different production systems result in different nutritional quality meats. Atthe store, the consumer may encounter intensively, semi-extensively and extensively grown beef. However, extensively grown beef is largely rarer to be found in supermarket butchery.

The probability of buying leaner meat with healthier fatty acids is higher if the consumer chooses semi-extensively or extensively grown meat.This work analysed health indicators such as thrombogenicity and atherogenicity indices for the first time, and it was verified that there are differences among production systems regarding these indices. In general, semi-extensive systems seems to lead to better nutritional quality than the intensive system. So, this led to the conclusion that the semi-extensive production system (used in Alentejo, Portugal) is a good way to achieve a good balance between environmental sustainability, beef quality and animal welfare.

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References

Afshari R, Hosseini H, Khaneghah AM & Khaksar R. 2017. Physico-chemical properties of functional low-fat beef burgers: Fatty Acid Profile Modification. LWT - Food Science and Technology, 78, 325-331.

Azain MJ, Hausman DB, Sisk MB, Flatt WP & Jewell DE. 2000. Dietary Conjugated Linoleic Acid Reduces Rat Adipose Tissue Cell Size Rather than Cell Number. The Journal of Nutrition, 130, 1548-1554.

Brand-Williams W, Cuvelier ME & Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28, 25-30.

Claeys E, Uytterhaegen L, Buts B & Demeyer D. 1995. Quantification of beef myofibrillar proteins by SDS-PAGE. Meat Science 39, 177-193.

Couvreur S, Le Bec G, Micol D & Picard B. 2019. Relationships Between Cull Beef Cow Characteristics, Finishing Practices and Meat Quality Traits of Longissimus thoracis and Rectus abdominis. Foods, 8, 141.

Daley CA, Abbott A, Doyle PS, Nader GA & Larson S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal, 9, 10.

de Alba M, Pérez-Andrés JM, Harrison SM, Brunton NP, Burgess CM & Tiwari BK. 2019. High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets. Innovative Food Science & Emerging Technologies, 55, 80-87.

De la Fuente J, Díaz MT, Álvarez I, Oliver MA, Font i Furnols M, Sañudo C, Campo MM, Montossi F, Nute GR & Cañeque V. 2009. Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. Meat Science, 82, 331-337.

Delosière M, Durand D, Bourguet C & Terlouw EMC. 2020. Lipid oxidation, pre-slaughter animal stress and meat packaging: Can dietary supplementation of vitamin E and plant extracts come to the rescue? Food Chemistry, 309, 125668.

Descalzo AM, Insani EM, Biolatto A, Sancho AM, García PT, Pensel NA & Josifovich JA. 2005. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. Meat Science, 70, 35-44.

Dhiman TR, Nam SH & Ure AL. 2005. Factors Affecting Conjugated Linoleic Acid Content in Milk and Meat. Critical Reviews in Food Science and Nutrition, 45, 463-482.

Enser M, Scollan ND, Choi NJ, Kurt E, Hallett K & Wood JD. 2016. Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. Animal Science, 69, 143-146.

Font i Furnols M, Realini C, Montossi F, Sañudo C, Campo MM, Oliver MA, Nute GR & Guerrero L. 2011. Consumer's purchasing intention for lamb meat affected by country of origin, feeding system and meat price: A conjoint study in Spain, France and United Kingdom. Food Quality and Preference, 22, 443-451.

García-Torres S, López-Gajardo A & Mesías FJ. 2016. Intensive vs. free-range organic beef. A preference study through consumer liking and conjoint analysis. Meat Science, 114, 114-120.

Gião M, González-SanJosé ML, Rivero-Perez M, Pereira C, Pintado M & Xavier Malcata F. 2007. Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenol content on extraction features. Journal of the Science of Food and Agriculture, 87, 2638-2647.

Heck RT, Vendruscolo RG, de Araújo Etchepare M, Cichoski AJ, de Menezes CR, Barin JS, Lorenzo JM, Wagner R & Campagnol PCB. 2017. Is it possible to produce a low-fat burger with a healthy n− 6/n− 3 PUFA ratio without affecting the technological and sensory properties? Meat Science 130, 16-25.

Hunt MR, Legako JF, Dinh TTN, Garmyn AJ, O'Quinn TG, Corbin CH, Rathmann RJ, Brooks JC & Miller MF. 2016. Assessment of volatile compounds, neutral and polar lipid fatty acids of four beef muscles from USDA Choice and Select graded carcasses and their relationships with consumer palatability scores and intramuscular fat content. Meat Science 116, 91-101.

Insani EM, Eyherabide A, Grigioni G, Sancho AM, Pensel NA & Descalzo AM. 2008. Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. Meat Science 79, 444-452.

Joo ST, Kauffman RG, Kim BC & Park GB. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Science, 52, 291-297.

Mahayothee B, Koomyart I, Khuwijitjaru P, Siriwongwilaichat P, Nagle M & Müller J. 2016. Phenolic Compounds, Antioxidant Activity, and Medium Chain Fatty Acids Profiles of Coconut Water and Meat at Different Maturity Stages. International Journal of Food Properties, 19, 2041-2051.

Mahecha L, Nuernberg K, Nuernberg G, Ender K, Hagemann E & Dannenberger D. 2009. Effects of diet and storage on fatty acid profile, micronutrients and quality of muscle from German Simmental bulls. Meat Science, 82, 365-371.

Menezes LFGD, Segabinazzi LR, Restle J, Freitas LdS, Brondani IL, Silveira MFD, Pacheco RF, Paula PCD & Joner G. 2013. Meat lipid profile of steers finished in pearl millet pasture with different rates of concentrate. Pesquisa Agropecuária Brasileira, 48, 553-558.

Mestre Prates JA, Gonçalves Quaresma MA, Branquinho Bessa RJ, Andrade Fontes CMG & Mateus Alfaia CMP. 2006. Simultaneous HPLC quantification of total cholesterol, tocopherols and β-carotene in Barrosã-PDO veal. Food Chemistry, 94, 469-477.

Mezgebo GB, Monahan FJ, McGee M, O'Riordan EG, Richardson IR, Brunton NP & Moloney AP. 2017. Fatty acid, volatile and sensory characteristics of beef as affected by grass silage or pasture in the bovine diet. Food Chemistry, 235, 86-97.

Mir PS, McAllister TA, Scott S, Aalhus J, Baron V, McCartney D, Charmley E, Goonewardene L, Basarab J, Okine E, Weselake RJ & Mir Z. 2004. Conjugated linoleic acid–enriched beef production. The American Journal of Clinical Nutrition, 79, 1207S-1211S.

Neel JPS, Fontenot JP, Clapham WM, Duckett SK, Felton EED, Scaglia G & Bryan WB. 2007. Effects of winter stocker growth rate and finishing system on: I. Animal performance and carcass characteristics1,2. Journal of Animal Science, 85, 2012-2018.

Nuernberg K. 2009. 14 - Optimising the nutritional profile of beef. *In*: Kerry, J.P., Ledward, D. (Eds.), Improving the Sensory and Nutritional Quality of Fresh Meat. Woodhead Publishing, pp. 321-341.

Pestana JM, Costa ASH, Alves SP, Martins SV, Alfaia CM, Bessa RJB & Prates JAM. 2012. Seasonal changes and muscle type effect on the nutritional quality of intramuscular fat in Mirandesa-PDO veal. Meat Science, 90, 819-827.

Pietrzak M, Greaser M & Sosnicki A. 1997. Effect of Rapid Rigor Mortis Processes on Protein Functionality in Pectoralis Major Muscle of Domestic Turkeys. Journal of Animal Science, 75, 2106-2116.

Pimentel LL, Fontes AL, Gomes AM & Rodriguez-Alcala LM. 2015. Considerations about the *in situ* derivatization and fractionation of EFA and NEFA in biological and food samples. Methods X2, 475-484.

Popova T, Ignatova M, Petkov E & Stanišić N. 2016. Difference in fatty acid composition and related nutritional indices of meat between two lines of slow-growing chickens slaughtered at different ages. Arch. Anim. Breed., 59, 319-327.

Qualidade IPd. 1979. NP 1613:1979 - Carnes, derivados e produtos cárneos. Determinação da matéria gorda total. Método de referência. Direcção-Geral da Qualidade.

Qualidade IPd. 2002. NP 1615:2002 - Carnes e produtos cárneos. Determinação da cinza total total. Método de referência. Instituto Português da Qualidade.

Qualidade IPd. 2006. NP 1612:2006 - Carnes e produtos cárneos. Determinação do teor de azoto total. Método de referência. Instituto Português da Qualidade.

Qualidade IPd. 2008. NP 3441:2008 - Carnes e produtos cárneos. Medição do pH. Método de referência. Instituto Português da Qualidade.

Qualidade IPd. 2009. NP 1614-1:2009 - Carnes e produtos cárneos. Determinação do teor de humidade. Parte 1: Método de referência. Instituto Português da Qualidade.

Ribeiro TB, Campos D, Oliveira A, Nunes J, Vicente AA & Pintado M. 2021. Study of olive pomace antioxidant dietary fibre powder throughout gastrointestinal tract as multisource of phenolics, fatty acids and dietary fibre. Food Research International, 142, 110032.

Ribeiro TB, Oliveira A, Coelho M, Veiga M, Costa EM, Silva S, Nunes J, Vicente AA, Pintado M. 2020. Are olive pomace powders a safe source of bioactives and nutrients? Journal of the Science of Food and Agriculture.

Romero MC, Romero AM, Doval MM, Judis MA. 2013. Nutritional value and fatty acid composition of some traditional Argentinean meat sausages. Food Science and Technology, 33, 161-166.

Schnettler MB, Vidal MR, Silva FR, Vallejos CL, Sepúlveda BN. 2008. Consumer Perception of Animal Welfare and Livestock Production in the Araucania Region, Chile. Chilean Journal of Agricultural Research, 68, 80-93.

Scollan ND, Dannenberger D, Nuernberg K, Richardson I, MacKintosh S, Hocquette JF & Moloney AP. 2014. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science, 97, 384-394.

Simopoulos AP. 2016. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients, 8, 128.

Sonoda Y, Oishi K, Chomei Y & Hirooka H. 2018. How do human values influence the beef preferences of consumer segments regarding animal welfare and environmentally friendly production? Meat Science, 146, 75-86.

Swain M, Blomqvist L, McNamara J & Ripple WJ. 2018. Reducing the environmental impact of global diets. Science of The Total Environment, 610-611, 1207-1209.

Teixeira RFM, Proença V, Crespo D, Valada T & Domingos T. 2015. A conceptual framework for the analysis of engineered biodiverse pastures. Ecological Engineering, 77, 85-97.

Tonsor GT, Olynk N & Wolf C. 2015. Consumer Preferences for Animal Welfare Attributes: The Case of Gestation Crates. Journal of Agricultural and Applied Economics, 41, 713-730.

Tornberg E. 2005. Effects of heat on meat proteins – Implications on structure and quality of meat products. Meat Science, 70, 493-508.

Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S & Ezaki O. 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes, 49, 1534.

Ulbricht T, Southgate D. 1991. Coronary heart disease: seven dietary factors. The Lancet, 338, 985-992.

Van Wezemael L, Verbeke W, de Barcellos MD, Scholderer J & Perez-Cueto F. 2010. Consumer perceptions of beef healthiness: results from a qualitative study in four European countries. BMC Public Health, 10, 342-342.

Vestergaard M, Therkildsen M, Henckel P, Jensen LR, Andersen HR & Sejrsen K. 2000. Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. Meat Science, 54, 187-195.

Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR & Enser M. 2004. Effects of fatty acids on meat quality: a review. Meat Science, 66, 21-32.